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BRIEF SUCCESSIVE TEMPORAL OBSERVATIONAL SAMPLING AS A POSSIBLE INDICATOR OF DAILY OVERT SEIZURE ACTIVITY IN EPILEPTIC RATS[1]

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Summary.—Chronic, limbic epilepsy was induced in male rats by a single systemic injection of lithium and pilocarpine. In two separate experiments, each 6 mo. in duration, the proportion of the population that displayed an overt motor seizure (head nodding, rearing, and forelimb clonus) within 10 min. each day after delivery of food pellets (despite food provided *ad libitum*) was recorded. Cumulative records for the numbers of rats in each population that displayed overt seizure for the first time during the observation period were plotted as a function of time. The occurrence of the saturated (approaching 100%) asymptote at about five months was interpreted to indicate that brief (10 min.) sampling over the 144 successive days that would compose a single 24-hr. period could serve as an estimate of the average probability of an overt seizure during an average 24-hr. period. If this assumption is correct, then these rats would have displayed about one overt seizure per day.

An intrinsic assumption of observation is that the characteristics of a single (cross-sectional) optimal sampling from a total population should reflect the characteristics of the average single element from that population were it followed longitudinally from its origin to its termination. For example, if a single, optimal cross-sectional sample was taken randomly from the entire human population, the seriation of these values should reflect the characteristics of a single, average person were he systematically measured (longitudinally) from birth to death.

The displays of paroxysmal, stereotyped behaviours in rats due to chronic limbic epilepsy induced by a single injection of lithium and pilocarpine are closely analogous to the brief automatisms that accompany complex partial (limbic) epilepsy in human beings (Persinger, Makarec, & Bradley, 1988). These transients, however, are brief and would require 24-hr. surveillance to document their incidence. If the conditions that precipitate these events occurred systematically during the 24-hr. day, then repeated observations of the same population of rats for 10 min. per day for several months should approximate the incidence rate for a single average epileptic rat that was measured continuously for 24 hr.

In Exp. 1 there were 32 male Wistar rats while in Exp. II there were 26

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male Sprague-Dawley rats (Charles River, Quebec). The two strains were used because they were available. All rats had been between 80 and 90 days of age at the time the seizures were induced by a single systemic injection of lithium (3 mEq/kg) followed 4 hr. later by a subcutaneous injection of 30 mg/kg of pilocarpine. Within one hour after the injection of the muscarinic agent, each rat received, subcutaneously, 25 mg/kg of acepromazine (Atravet) to facilitate survival.

After approval by the local Animal Care Committee, the experiments began about two months after the induction of the chronic epilepsy. In Exp. I (Wistar, October, 1993 through March, 1994) and in Exp. II (Sprague-Dawley, October, 1994 through March, 1995), each rat was housed singly within the same standard cage rack within a temperature controlled (19° to 21° C) room. Whereas the rats in Exp. I had been housed in groups of three before they were placed in single cages, the rats in Exp. II had been housed singly since the induction of the seizures.

The light-dark cycle was 12:12 with the night cycle beginning at 20:00 hr. Food (Purina rat chow) and water were available *ad libitum*. Between 19:15 and 19:30 hr. each day in Exp. I and between 18:00 hr. and 19:00 hr. each day in Exp. II, two cubes of rat chow were placed in each rat's cage (total time required to add food to all cages < 1 min.). This test time was selected to allow potential comparisons with previous studies (Persinger, 1995a).

The occurrence of limbic overt seizures, as defined by rearing, rapid forelimb clonus, and falling, equivalent to kindling Stage 5 (Michael, Holsinger, Ikeda-Douglas, Cammisuli, Ferbinteanu, DeSouza, DeSouza, Fecteau, Racine, & Milgram, 1998), within the first 10 min. after introduction of the food was recorded for each rat. The occurrence of these overt displays is extremely conspicuous and specific. Consequently there has been 100% concordance between the tens of senior neuroscience students who have participated in previous and subsequent studies.

During the 6 mo. of daily observations, 7 rats (22%) died in Exp. I and 6 (23%) rats died in Exp. II. This mortality is consistent with previous observations concerning sudden unexpected death in epileptic rats (Persinger, 1995b) and with the precipitation of seizures and subsequent lateral hypothalamic-like syndrome (hypophagia, shift in palatability of foods, and weight-loss) that occur after one or two days of global geomagnetic activity exceeding 40 nT. The mean numbers of daily seizures per month for the rats in the two samples that survived for 6 mo. ($n = 25$ in Exp. I, $n = 20$ in Exp. II) were analyzed by two-way analysis of variance with one level repeated (month) and one not repeated (experiments).

Two-way analysis of variance with one level repeated (months) demonstrated a statistically significant difference ($F_{1,43} = 9.06$, $p < .01$; $\eta^2 = 17\%$) be-

tween the proportion of daily seizures per month for the rats in Exp. I (Wistar) compared to Exp. II (Sprague-Dawley). There was, however, no statistically significant difference ($F_{5,215} = 0.51$, $p > .05$) over time (between months); the interaction between time and the two groups was not statistically significant ($F_{5,215} = 1.88$, $p > .05$). The means and standard deviations for the percentages of rats within each population that displayed overt seizures per day for each month are shown in Table 1.

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR PERCENTAGE OF POPULATION OF CHRONIC EPILEPTIC RATS THAT EXHIBITED OVERT LIMBIC SEIZURES (FOREPAW CLONUS AND REARING) PER DAY WITHIN 10 MIN. OF INTRODUCING A SYNCHRONIZED FOOD STIMULUS*

Month	Experiment I		Experiment II	
	M	*SD*	*M*	*SD*
October	1.5	2.4	4.8	5.2
November	1.5	2.2	6.6	8.3
December	2.3	2.2	4.3	4.9
January	1.5	4.5	4.1	5.1
February	3.3	4.4	5.6	7.1
March	3.5	4.5	4.6	5.2

*Exp. I and Exp. II involved two separate years.

Inspection of the daily records (Fig. 1) indicated that 94% of the population in Exp. I (all but one) and 100% of the population in Exp. II exhibited at least two overt seizures (the total numbers of episodes of forelimb clonus for each rat ranged from 2 to 25) during the 6-mo. periods. The cumulative percentages of the different rats that exhibited an overt seizure for the *first* time during the 10-min. observations periods saturated within a few days of 5 mo. for both experiments.

There are 144 10-min. periods in a day. Assuming a random occurrence of the processes that encourage the display of overt seizures with respect to the fixed 10-min. interval, one would expect the cumulative number of rats that displayed at least one overt seizure during the protracted interval to reflect the average rate of daily seizures. Both groups of rats achieved near-saturated asymptote within about 5 mo. If this concept be valid, then the chronic limbic epilepsy induced by a single systemic injection of lithium and pilocarpine results in the overt display of paroxysmal forelimb motor movements on the average of approximately once per day. Direct comparison of these data with recordings from experimenters who have measured the numbers of spontaneous subclinical (nonovert) and overt paroxysmal episodes in rats seized by lithium and pilocarpine would support the validity of this approach.

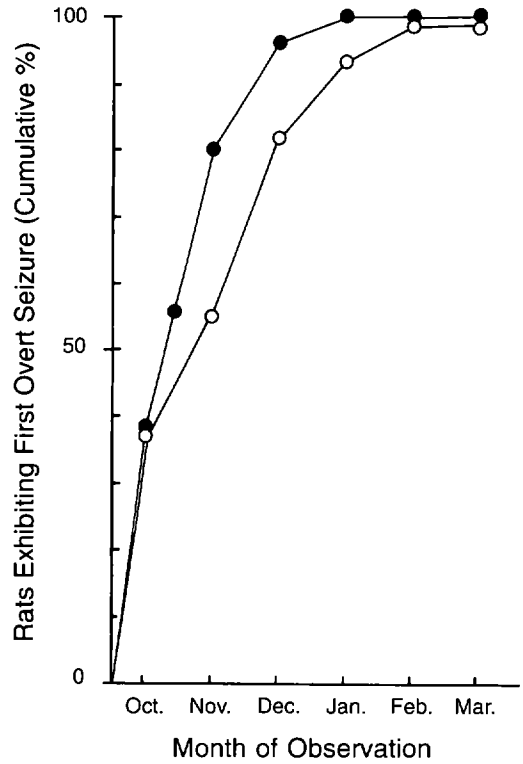

FIG. 1. Cumulative percent of rats exhibiting Racine Level 5 (overt) seizures as a function of time in month. Exp. I (o), Exp. II (•)

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